REMARKS

Claims 7, 9, 12, 13, 18, 20, 23, 26 and 27 are in the case. Claim 12 has been amended in view of the Office Action and to better define what Applicants consider their invention.

Reconsideration in view of the following remarks and entry of the foregoing amendments are respectfully requested.

DOUBLE PATENTING

Claims 7, 9, 18, 20, 23 and 26-27 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 36 and 38 of copending Application No. 10/530,413 (hereinafter the '413 application).

The Examiner is of opinion that claims 7, 9 and 26 are generic to all that is recited in claim 36 of the '413 application and that, consequently, instant claims 18, 20, 23 and 27 are not patently distinct from the conflicting claims. This rejection is provisional since the conflicting claims have not in fact been patented.

Applicants duly noted this provisional rejection but do not wish to address it at this time. Applicants recognize that this objection may continue to be made by the Examiner in this application as long as she is of the opinion that there are conflicting claims in the two applications that are the subject of this rejection, subject to the following: "If this "provisional" non statutory obviousness-type double patenting (ODP) rejection is the only rejection remaining in the earlier filed of the two pending applications, while the latter is rejectable on other grounds, the Examiner should withdraw that rejection and permit the earlier filed application to issue as a patent without a terminal disclaimer" (MPEP 804 I.B.1).

As indicated previously, the present application is the earlier filed of the two applications that are the subject of the present rejection. Applicants therefore do not wish to address this issue in the present response since the provisional rejection may be withdrawn.

REJECTIONS UNDER 35 U.S.C. § 112 SECOND PARAGRAPH

Claims 12 and 13 are newly rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. This new rejection is necessitated by the cancellation of claim 11 without amending claims 12 and 13 to depend on a pending claim in the reply filed April 17, 2008.

As requested by the Examiner, claim 12 has been amended and now depends on claim 9.

REJECTION UNDER 35 U.S.C. § 103

Claims 7, 9, 18, 20, 23 and 26-27 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Largman *et al.*, and in view of Frankel *et al.* The arguments filed April 17, 2008 have been considered but were not found persuasive.

In Largman *et al.*, stem cells were modified by the incorporation of exogenous genetic material into their genome. The introduction of the exogenous genetic material is preferentially performed by retroviral infection. The modified stem cell overexpressing HOXB4 is characterized by an enhanced ability to undergo self-renewal as compared to unmodified stem cells.

Franckel *et al.* teach the delivery of biologically active proteins to the cytoplasm and nuclei by the use of transport polypeptides which comprise an HIV tat protein covalently attached to the cargo.

According to the Examiner, it would have been obvious to one of ordinary skill to modify the method of generating expanded populations of stem cells of Largman to "replace the delivery of HOXB4 protein by delivering a nucleic acid molecule with the delivery of HOXB4 protein by delivering a tat-conjugated protein as taught by Frankel *et al*". "One would have been motivated to make such a modification in order to receive the expected benefit of more efficiently delivering the HOXB4 protein to the nucleus of the cells as taught by Frankel et al. Based upon the teachings of the cited references, ***the high skill of one of ordinary skill in the art and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention***." The Examiner is thus of the

opinion that based on the teachings of the cited references, there would have been a reasonable expectation of success to result in the claimed invention.

Applicants respectfully disagree and traverse the rejection as follows.

Applicants first wish to state that since the United States Supreme Court in *KSR International Co.* vs. *Teleflex Inc. et al.* 550 U.S. 398 (2007) ("*KSR*") rejected the Court of Appeals' application of the teaching, suggestion, or motivation test because it was too rigid, it certainly did not intend to replace this rigid test with another rigid test where whenever is found a motivation to test a particular scientific avenue, then obviousness must be found. The Supreme Court rejected any notion that the concept of obviousness in patent law can be rigidly or narrowly defined holding that "the obviousness analysis cannot be confined by a formalistic conception." (page 15, second paragraph of *KSR*).

Justices Lourie, Newman and Rader in *Pfizer Inc.* vs. *Apotex* 82 USPQ 1852 (21 May 2007) also stated that:

> "[H]olding an inventor's expectations of success against the objective unexpectedness of the properties of the compound unfairly suggests that an inventor should try only that which he doubts will work. [...]. Inventors generally are optimistic about what they choose to experiment with, but that does not necessarily suggest obviousness." (paragraph bridging pages 13 and 14 of the decision).

Unpredictability

As indicated in Applicants' previous response and as supported by Dr. Humphries' Declaration, it was not routine experimentation to produce a functional stem cell expansion factor which comprised a HOXB4 protein and a NH_2-terminal protein transduction domain (PTD) from a transactivating protein (TAT). In addition, it was not predictable whether a functional HOXB4 non-gene delivery could be achieved.

Indeed, many factors influence the success of tat-fusion protein delivery and tat-fusion protein function. Non-limiting examples include: 1) cell type; 2) the fusion protein itself (which may affect protein folding, function, cellular localization, etc.); 3) the purification method of the TAT-fusion protein; and 4) the toxicity of the TAT-fusion protein. The influence of these

factors on the success of a particular tat-fusion protein is unpredictable and accordingly, no reasonable expectation of success can be predicted simply by looking at the structure of the particular tat-fusion protein pair. Each case must be tested and each experiment must overcome its specific obstacle before success can be achieved.

1. Cell type and subcellular localization

For example, the subcellular localization of transduced proteins may depend on cell type, nature of the imported proteins and delivery approach for a given TAT-fusion protein. Indeed, discrepancies have been observed between the subcellular localization of TAT-GFP transduced vs. transfected cells (Yang *et al.*,[1]-enclosed). Thus, one cannot predict whether a given TAT-fusion protein would display the right subcellular localization for proper functioning. Accordingly, one cannot predict whether the TAT-HOXB4 fusion protein would be localized properly into the cell in order to display appropriate expansion potential.

2. Toxicity, delivery and function

Whether a given TAT-fusion protein would be functional and whether it would be toxic is also unpredictable. For example, Reis *et al.*[2] (abstract enclosed), reported that the uptake of a TAT-FMRP (fragile X mental retardation protein) fusion protein was highly inefficient and uptake velocity lower than expected. In addition, the fusion protein was reported to be highly toxic to fibroblasts.

Moreover, El-Andaloussi *et al.*[3] (enclosed), studied cargo-dependent cytotoxicity and delivery efficacy of cell-penetrating peptides (CPPs, including TAT). This study showed that cellular uptake and cytotoxicity of CPPs depend on the particular fusion protein. In addition, the author states that this study "could be useful in future experiments utilizing CPPs to facilitate the choice of peptide depending on cargo and choice of conjugation strategy". Therefore, it appears that in 2007 (i.e., more than 4 years after the filing date of the present application), people skilled in the art of CPPs believed that not all CPPs, including TAT, combined with any type of protein in any type of cell would 1) be transduced efficiently; 2) be

[1] Yang *et al.*, 2002, FEBS Letters 532, pp. 36-44.
[2] Reis *et al.*, 2004, Journal of Molecular Histology, 35:389-395.
[3] El-Andaloussi et al., 2007, *Biochem J.* 407:285-292.

biologically active; and 3) not be toxic. This further highlights the fact that there exists a number of cell-penetrating peptides from which to choose and that one could not have predicted the success of stem cell expansion from a TAT-HOXB4 fusion protein. In addition, the fact that various techniques have been developed and continue to be developed since the discovery of the PTD domain in 1994 to transduce fusion protein into cells supports the idea that this technique is not as universal as it is alleged to be by the Examiner and that it may not work properly with several of the proteins to which it is fused.

The fact that the success of protein internalization is dependent on the particular TAT-fusion is further illustrated in El-Andaloussi *et al.* which state "Strikingly, TAT efficiently internalizes to cells when conjugated via biotin to proteins [i.e., avidin or biotin], but is poorly internalized as a fluorescein-coupled peptide compared with TP10 and penatratin (Figures 1a, 1b and 5c)" [emphasis added]. This suggests that depending on the particular molecule to which it is fused and how it is fused, the TAT-fusion molecule will not display the same internalization rate. Again, this further emphasizes the unpredictability of the art.

Moreover, Jones *et al.*[4] - enclosed -also reported that:

> *"**Peptide conjugation** had no significant effect upon the response to transportan (EC_{50}=5 uM), polyarginine (EC_{50} 12 uM) and antennapedia (EC_{50} 21 uM), **but increased the toxicity of TAT at 100 uM**, reducing the EC_{50} to 67 uM (Figure 2 - hatched lines). To examine the effect of rhodamine labelling and the biological action of PKI-derived peptide, we also undertook further studies in A549 cells using unlabelled CPPs (Figure 3 – solid lines) and those conjugated to a peptide derived from the NEMO-binding domain (NBD) (short hatched lines) and its scrambled control (Table 1) (Figure 3 – long hatched lines) (May et al., 2000). Interestingly, these studies suggested that rhodamine increased toxicity, since in the absence of the label, antennapedia, TAT and transportan alone gave no significant toxicity at concentrations up to 100 μM, while polyarginine gave 20 and 68% reductions in cell number at 30 and 100 μM, respectively. **The conjugation of either the NBD and scrambled peptide had no effect upon the antennapedia response, but increased the toxicity of TAT at the highest concentration (100 μM)** and reduced the EC_{50} with polyarginine from approximately 80 to 15 μM."* [Emphasis added] (Page 1096, left column, last paragraph to right column first paragraph); and

> *"**Recent attempts to characterise the intracellular mechanism have focused upon the delivery of protein conjugates by the TAT sequence***

[4] Jones et al., 2005, *British Journal of Pharmacology*, 145:1093-1102.

> ***and have produced contradictory conclusions...The reasons for these differences are unknown, but may relate to differences <u>in cell type or cargo</u>.*** *In addition, uptake may be influenced by the individual CPPs, since comparative studies have shown that the* ***<u>uptake of TAT but not antennapedia complexes are inhibited by dextran sulphate and that they localise to different intracellular compartments.</u>*** *"* [Emphasis added] (Console et al., 2003).

Spitere *et al.*[5] (abstract enclosed), reported no toxicity following TAT-PAX6 protein transduction in neural progenitor cells although TAT proteins and TAT fusions have been reported as toxic in neuronal cells (see for example Reis *et al.* enclosed). This further demonstrates that depending on the particular cargo protein, for a given cell type one TAT fusion may be toxic while the other may not, and further supports the unpredictability of the art. Thus, not only the cell type or the protein cargo alone may influence functional delivery but also the specific combination TAT-fusion protein and cell type is important. Ultimately, one cannot be sure whether a delivery will be successful even when the same cell type has been successfully transduced with a TAT-fusion protein or when the same TAT-fusion protein has been transduced in another cell type.

Falnes *et al.*[6], (abstract enclosed) reported that a fusion protein between dtA (diphteria toxin A-fragment) and TAT enabled the protein to bind avidly to the cell surface but did not allow for a biologically active fusion protein to be delivered into the cytosol.

> *"A number of proteins are able to enter cells from the extracellular environment, including protein toxins, growth factors, viral proteins, homeoproteins, and others. Many such translocating proteins, or parts of them, appear to be able to carry with them cargo into the cell, and a basic sequence from the HIV-1 TAT protein has been reported to provide intracellular delivery of several fused proteins. For evaluating the efficiency of translocation to the cytosol, this TAT-peptide was fused to the diphtheria toxin A-fragment (dtA), an extremely potent inhibitor of protein synthesis which normally is delivered efficiently to the cytosol by the toxin B-fragment.* ***<u>The fusion of the TAT-peptide to dtA converted the protein to a heparin-binding protein that bound avidly to the cell surface</u>****. However, no cytotoxicity of this protein was detected,* ***indicating that the TAT-peptide <u>is unable to efficiently deliver enzymatically active dtA to the cytosol</u>. Interestingly, the fused TAT-peptide potentiated the binding and cytotoxic effect of the corresponding holotoxin...The data indicate***

[5] Spitere *et al.*, 2008, Brain Res., 1208:25-34.
[6] Falnes *et al.*, 2001, Biochemistry, 40(14):4349-58.

that transport of dtA into the cell by the TAT-peptide and VP22 is inefficient". [Emphasis added]

Protein purification

In addition, protein purification is known to influence the transduction and biological activity of TAT fusions. Nagel *et al.*[7] (abstract enclosed), recently studied the effect of protein purification on biological activity of TAT-HSP70 fusion.

> "...the impact of the purification procedure on the functionality of TAT-fusion proteins has not been systematically examined [...] **[The result] suggests that the method of recombinant TAT-fusion protein purification influences its functionality.** For TAT-Hsp70, the method of choice seems to be isolation under native conditions, for which we present a purification protocol. Our results may contribute to improve TAT-fusion protein application in basic research and may facilitate its use as therapeutic tool, for instance in Parkinson's disease." [Emphasis added]

Finally and as indicated in Dr. Humphries's Declaration, Applicants reiterate that the successful transduction of a functional TAT-HOXB4 fusion protein was hampered by several obstacles such as methods of production, purification and storage, dosage (amount and frequency); *in vitro* conditions, and nature of starting cells that would respond. More than six months were necessary to generate the first TAT-HOXB4 fusion protein and a further 6 months was necessary to generate the first data showing that TAT-HOXB4 could induce HSC expansion. If the present invention required only routine experimentation and was as straightforward as alleged by the Examiner, a successful transduction would have been possible in a matter of weeks and with limited manpower. Conversely here, more than a year and significant resources have been required to arrive at the present invention.

Applicants would like to stress that routine experimentation should not be confused with available methods which often require further modifications and a lot of effort in order to find success. It is not because a method is available that it is necessarily routine. In addition, where several methods are available and where a person skilled in the art is faced with several alternatives, undue experimentation may be required to finally find a successful

[7] Nagel *et al.*, 2008, *Neurosci Methods*, 171(2):226-32.

alternative (albeit known). Moreover, Applicants would like to stress that "**Patentability shall not be negated by the manner in which the invention was made**" (second sentence of 35 U.S.C. 103- and...). In addition, and as stated in Re Deuels (51 F.3d 1552, FC 1993) "A general incentive does not make obvious a particular result, **nor does the existence of techniques by which those efforts can be carried out**."

Long-felt but unresolved need

The Examiner states that since methods providing HSC for transplantation to patients without an HLA matched donor existed prior to the method of the present invention, (i.e., 1) transplantation of umbilical cord blood isolated stem cells; 2) transplantation of mismatched grafts, and 3) autologous transfer of mobilized stem cells), then there is no "long felt but unresolved need". The need was satisfied by these methods of the prior art.

Applicants respectfully disagree with this conclusion.

Applicants first submit that the long-felt need in the present case would be better characterized as a need for a method that would be able to significantly increase the number of patients who truly have access to successful HSC transplantation, and in particular those who do not find an HLA matched donor (see paragraph 8 of the Declaration submitted April 17, 2008).

For these patients, the above-mentioned options of transplanting umbilical cord blood isolated stem cells, transplanting mismatched grafts or autologous transfer of mobilized stem cells have been known for several years. Although the above three methods may provide some relief to certain patients, they comprise important limitations and disadvantages which translate into many patients getting unsuccessful transplantation (leading to death) or suffering from life-threatening side effects. Thus, it has been long recognized that there remains a need for improvements in the above methods to effectively and successfully treat all patients that are in need of HSC transplantation.

For example, limited umbilical cord blood (UCB) cell dose compromise the outcome of adult patient UCB transplantation. In general UCB transplantation's outcome is correlated with the cell dose infused. Although UCB transplantation for children with hematopoietic malignancies has become standard therapeutic, it is severely limited in adult by

graft cell dose. Indeed, only about 30% of adult referrals to the University of Minnesota were eligible for UCB transplantation on the basis of a single unit of cord blood with an adequate cell dose. Further, of those patients who proceeded to transplantation, only 72% engrafted if the infused cell dose was less than 1.7 $x10^5$ CD34/Kg (Wagner JE, et al., Blood 2002; 100, 1611-1618). In addition, while the limited graft cell dose may possibly be overcome by transplanting multiple umbilical cord blood, it increases the risk of infection, increases the number of manipulations necessary and requires that sufficient cord blood samples be available for transplantation. In addition, recipients that get higher cell doses have significantly more rapid recovery as compared to those of lower cell doses. Also, some studies suggest that graft-versus-graft reactions occur leading to single-donor predominance. With regard to transplantation of mismatched grafts, this procedure remains very complicated and is not offered to all patients as it is still considered an experimental treatment. Patient's T lymphocytes need to be completely eliminated prior to the treatment and then the immune system must be replenished. These patients are more at risk of suffering from dangerous infections and older patients are not offered such treatment. In addition, it is sometimes difficult to obtain a sufficient amount of stem cells, even in the case of mismatched grafts. Finally, autologous transfer of mobilized stem cells is only available for a very limited number of patients which have healthy stem cells in a sufficient amount. Patients suffering from congenital illnesses, leukemia, bone marrow aplasia or any treatment-resistant form of blood related illnesses cannot benefit from autologous transfer of mobilized stem cells.

Thus, it is clear from the above, that even if treatment is available, there remains a need to improve such treatments since for many patients, they are either not available or unsuccessful.

The above-noted options are clearly inadequate since mortality is still very significant today although the methods have been known for years. Applicants submits that it would be inappropriate to conclude that a long-felt need in the present case can only be demonstrated by showing that no options at all were available for these patients where the available options are clearly inadequate to significantly reduce the mortality.

Applicants submit that all of the above available treatments would benefit from the present invention because it provides a way to increase the number of stem cells available

for a transplantation in UCB transplantation as well as in mismatched grafts and autologous grafts. For Example, a single UCB unit could be treated with a TAT-HOXb4 protein to increase the stem cell population such that a greater number of adult patients would be eligible to UCB transplantation. Similarly, expansion of stem cell population using TAT-Hoxb4 in mismatched grafts would help to successfully treat a greater number of patients, require less graft and may even decrease susceptibility to infections since by transplanting a greater number of stem cells, the patient would be immunodeficient for a shorter amount of time. Also, it has been recognized that higher cell dose may partially overcome the negative impact of certain histocompatibility leukocyte antigen (HLA) disparities in UCB transplantation (see Schoemans et al.[8], Bone Marrow (2006) 38, 83-93, enclosed). Even autologous transplantation could benefit from the present invention. Indeed, cancer patients which have a very depleted stem cell population due to cancer treatment and which generally cannot take advantage of autologous transplantation could have their stem cell population increased sufficiently such that autologous transplantation is now possible.

Section 716.04 of the MPEP states that long-felt but unresolved need can be established as follows:

> "The relevance of long-felt need and the failure of others to the issue of obviousness depends on several factors. **First, the need must have been a persistent one that was recognized by those of ordinary skill in the art.** *In re Gershon*, 372 F.2d 535, 539, 152 USPQ 602, 605 (CCPA 1967) ("Since the alleged problem in this case was first recognized by appellants, and others apparently have not yet become aware of its existence, it goes without saying that there could not possibly be any evidence of either a long felt need in the art for a solution to a problem of dubious existence or failure of others skilled in the art who unsuccessfully attempted to solve a problem of which they were not aware."); *Orthopedic Equipment Co., Inc. v. All Orthopedic Appliances, Inc.,* 707 F.2d 1376, 217 USPQ 1281 (Fed. Cir. 1983) (Although the claimed invention achieved the desirable result of reducing inventories, there was no evidence of any prior unsuccessful attempts to do so.).
>
> **Second, the long-felt need must not have been satisfied by another before the invention by applicant.** *Newell Companies v. Kenney Mfg. Co.*, 864 F.2d 757, 768, 9 USPQ2d 1417, 1426 (Fed. Cir. 1988) (Although at one time there was a long-felt need for a "do-it-yourself" window shade material which was adjustable without the use of tools, a prior art product fulfilled the

[8] Schoemans et al., Bone Marrow Transplantation (2006) 38:83-93

need by using a scored plastic material which could be torn. "[O]nce another supplied the key element, there was no long-felt need or, indeed, a problem to be solved".)

Third, the invention must in fact satisfy the long-felt need. *In re Cavanagh*, 436 F.2d 491, 168 USPQ 466 (CCPA 1971)." [emphasis added]

Applicants submit that the three elements of this test are satisfied:

First, the need has been shown to be a persistent one that was recognized by those of ordinary skill in the art. As indicated above, at the time the present invention was made it had been long recognized that a great number of patients were either unsuccessfully treated or did not have access to these treatments at all (*e.g.*, large or tall patients (insufficient number of cells/kg in the sample); older patients etc.). In addition, in the case of mismatched graft enhancement or transplantation of multiple cord blood units, patients are more susceptible to infections, which has in many case compromised success of transplantation. Thus, there was still a need for a generally available, successful HSC stem cell transplantation treatment.

Second, the long-felt need must not have been satisfied by another before the invention by applicant. As indicated above, there still remains a very important number of adult patients for which stem cell transplantation is either unsuccessful or unavailable. Mortality of patients without an HLA matched donor is still too high despite the existence of the three above-mentioned methods. Thus, they cannot be said to "satisfy" the long-felt need for a significant increase of the number of patients who have access to successful HSC transplantation.

Third, the invention must in fact satisfy the long-felt need. As indicated above and in the Declaration submitted April 17, 2008, the method of the present invention would be able to derive a sufficient amount of HSCs for transplantation from a single umbilical cord blood sample. It could also be applied on HLA-mismatched grafts to enrich the samples in HSCs and to decrease susceptibility to infections. Even autologous transplantation would benefit from the present invention by allowing cancer patients to access to the safer autologous transplantation. The method of the invention would therefore significantly increase the number of patients who have access to HSC transplantation and resolve a long felt need. (see

paragraph 8 of the Declaration.)

Commercial success

The Examiner is of the opinion that evidence of commercial success was not provided because the claimed invention is not commercially available.

Applicants respectfully disagree and submit the following.

In the life science field, "commercial success" can also be measured by the level of funding a particular project is given by public and private instances especially when the project is at the stage of entering clinical trials which are extremely costly. Projects which already show little expectation of success or which do not fulfill a sufficient need are denied funding while projects which provide new and improved treatment methods and fulfill an important need are supported. Thus, a strong measure of peer recognition is success in peer reviewed grant competitions for applications that include research into investigations of TAT-HoxB4 production/delivery and efficacy. The present inventors have indeed been granted a number of grants totalizing several millions of dollars to pursue TAT-HoxB4 investigations and to develop the claimed invention. These include:

- NIH (USA) RO1 HL065430-06 "HOB4 is an activator of HSC self-renewal" funding period May 2005 to April 2010. Total award $1,600,000.
- CIHR (Canadian Institute of Health Research) Proof of Principle grant "Novel agents for hematopoietic stem cell expansion" funding period Sept 2005-2006. $120,000.
- CIHR "Team Grant on Stem Cell Expansion" funding from 2006 to 2011. Total award $4000,000.
- Canadian Stem Cell Network catalyst grant on "Production of TAT-HOX fusion protein" funding from 2006-2007. $50,000; and
- FRSQ (Fédération de Recherche en Santé du Québec). Total award 900,000$ (300,000/year from May 2007 to May 2010).

Overall, the above demonstrate the unpredictability of the art and support the finding that there was no reasonable expectation of success of fusioning the TAT PTD fragment to the HOXB4 protein to efficiently deliver the protein into stem cells and also to provide a functional fusion protein that would display stem cell expansion activity. Accordingly, Applicants submit that claims 7, 9, 18, 20, 23 and 26-27 are inventive and respectfully requests that the Examiner withdraw the rejection under 35 U.S.C. 103(a).

The rejections of the original claims are believed to have been overcome by the present amendments and remarks. From the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order, and such an action is earnestly solicited.